

[Reprinted from TEXAS REPORTS ON BIOLOGY AND MEDICINE, Volume 17,
Number 1, pages 49-59, Spring, 1959]

SOME ECOLOGICAL ASPECTS OF VITAMIN B₁₂-ACTIVE SUBSTANCES

THEODORE J. STARR^a AND FRED SANDERS

Introduction

Microorganisms occupy a singularly important position in study of marine ecology because of their widespread distribution and diverse physiological properties (56, 61, 63). To a significant degree, they govern the productive capacity and fertility of the oceans and estuarine environments. From the algal phototrophs which form the basis of the food-network through the heterotrophic bacteria which participate in the production and transformation of organic matter, there is a series of interrelated biological processes affecting the succession of populations. This sequence of events serves to enrich or deplete the waters of soluble and insoluble substances which may be essential (*i.e.*, vitamin B₁₂-active substances) or at times toxic (*i.e.*, toxins of algal flagellates) to life. The extent of biological change is affected in turn by physical and chemical factors. Water movements aid in the transport, concentration, and dilution of these substances which are formed as a result of microbial activities. In addition, microbial products may be added to the environment by the continual flushing action of tidal waters as they flood and ebb from the land. The results of these combined processes may be beneficial or detrimental to distant as well as neighboring communities.

Catastrophic mortalities of marine inhabitants associated with massive blooms of certain algal flagellates (10, 20, 60) illustrate the detrimental effects of the toxins (2, 38, 45, 49) produced by some members of this group. Specifically, the massive fish kills accompanying sudden blooms of *Gymnodinium breve* (12) suggest the following as yet hypothetical situation which exemplifies the interrelations between organisms in the marine environment. Briefly, the growth of *G. breve* may be stimulated to concentrations which are toxic to fish and other marine life by vitamin B₁₂ (59), a product of microbial origin (6, 17, 51). Vitamin B₁₂ produced in a particular locality, otherwise unsuitable for the

growth of *G. breve*, may be transported by water movements (currents, tides, water masses) to vitamin B₁₂-deficient areas that do have the other necessary physical and chemical attributes. Further studies are necessary to affirm whether vitamin B₁₂ or other essential micronutrients are limiting factors in potential "bloom" areas. Analogous processes have been cited by Lucas (31) who reviewed evidence in support of his concept of non-predatory relationships between organisms in aquatic environments and by Lochhead (28) and Starkey (46) in their recent reviews on some aspects of soil microbiology.

Among the many beneficial metabolic processes of microorganisms which illustrate their ecological importance are 1) photosynthesis by algae, 2) the physiological activities of bacteria in the carbon, nitrogen, sulfur, and phosphorus cycles, 3) the degradation of plant and animal residues by bacteria, and 4) the synthesis of organic matter, essential micronutrients, and vitamins.

The chemistry and functions of vitamin B₁₂ in the metabolism of microorganisms and animals have been reviewed extensively (19, 23, 35, 58) since its simultaneous isolation by teams in United States and England. Today, vitamin B₁₂ represents a family of compounds, members of which are essential for the normal growth and development of various organisms. For example, only the naturally occurring cyanocobalamin and factor III (3, 41), "true vitamins B₁₂," are clinically active against pernicious anemia in man. Some microorganisms have less specific requirements and can use one or more of the many vitamin B₁₂-analogues (19, 26). Various investigators have reported on the stimulatory or essential nature of cobalamin (vitamin B₁₂) for the *in vitro* growth of cultures of marine algae (14, 25, 27, 54, 59). Droop (16) and co-workers tested fourteen species of marine algae and demonstrated several specificity patterns. They suggested that the analogues of vitamin B₁₂ may have an importance equal to that of the vitamin. Are these vitamins, at times, limiting factors in the marine environment is a question which should be answered. However, the accumulated evidence illustrates a vital ecological role of vitamins (37) in the economy of the oceans, in the production of algal blooms, and in the succession of populations.

In this communication, several aspects of the vitamin B₁₂ cycle

are discussed to illustrate 1) the probable ecological importance of specific microbial activity, 2) a biological process which may influence the succession of microbial, invertebrate, and vertebrate populations, and 3) to emphasize the continued need for extensive cooperation between the basic physical, chemical, and biological sciences whose efforts are directed toward a better understanding of the oceans from both a fundamental and applied point of view. Part of the vitamin B₁₂ cycle was studied from the standpoint of production and biosynthesis by bacteria, distribution in oceanic and estuarine waters, and its occurrence in the sera of fish.

Production of vitamins B₁₂ by bacteria

Vitamins B₁₂ are products mainly of microbial origin (7, 21, 30). In experiments conducted under the auspices of the U.S. Fish and Wildlife Service, production of vitamin B₁₂-active substances by cultures of marine bacteria (51) was measured by microbiological assay procedures with *Escherichia coli* 113-3 and *Euglena gracilis*, z strain. Differential growth responses of *E. coli* and *Euglena* to members of the naturally occurring vitamins B₁₂ have been described in detail (9, 19, 24). Briefly, *E. coli* has a broader spectrum of response than *Euglena*. Growth of *E. coli* is stimulated by methionine and factor B, whereas *Euglena* does not respond to either of them. Of 34 newly isolated bacterial cultures (50), 30 per cent and 70 per cent had activity for *Euglena* and *E. coli*, respectively. These data are in agreement with those of Ericson and Lewis (17) who screened 34 cultures by the *E. coli* 113-3 cup-plate assay and found that 70 per cent were producers. Burton and Lochhead using the *Lactobacillus lactis* assay (6) found that 65 per cent of their terrestrial isolates were producers. A table describing the responses of various assay organisms to vitamin B₁₂-active compounds is given in Ford and Hutner (19).

In all cases in which aliquots of the same culture were tested with both organisms, the *E. coli* assay gave decidedly higher values. The maximum levels of vitamin B₁₂-activity were 5.00 and 18.4 mμg/ml for *Euglena* and *E. coli*, respectively. In the experiments of Burton and Lochhead (6), terrestrial bacteria produced over 500 mμg activity per ml of culture medium. The production of up to 150 mμg/ml (5) and 0.005-140 mμg/ml (17)

by marine bacteria has been reported. Ericson and Lewis (17) state that a level of approximately 10 $\mu\text{g}/\text{ml}$ is the more consistent value obtained with bacteria isolated from seawater and seaweed. The latter figure agrees with the amounts found during these studies.

In experiments designed to determine if vitamin B_{12} -activity was confined to the bacterial cell residues or released into the medium, results varied with each culture (51). Generally, intracellular levels were higher than extracellular. No apparent relationship was evident between the activity of either the cell residues or supernatants and the amount of bacterial growth or final pH of the medium.

The results of differential B_{12} -assays of bacterial cultures suggest a preponderance of vitamin B_{12} -analogues in nature compared to "true vitamins B_{12} ." In addition, these observations indicate that the biosynthesis occurring in nature is dependent on species specificity and availability of essential metabolites.

Directed biosynthesis of vitamins B_{12}

Cyanocobalamin is a water-soluble, relatively heat-stable vitamin of high molecular weight (39). Hydrolysis of the substance releases the two major components: factor B, a cobalt-containing porphyrinoid chromophore, and a nucleotide that contains the base 5, 6-dimethylbenzimidazole (1, 22). More than a dozen analogues of cyanocobalamin that occur in nature have been recognized (26). While all members of the group contain factor B, each is distinguished by the nitrogenous base which confers biological specificity (4). Only the "true" vitamins B_{12} , cyanocobalamin (57) and factor III (3, 41), are effective in the management of pernicious anemia. In contrast, the "pseudo"-vitamins B_{12} are clinically ineffective, although they do meet the vitamin B_{12} requirements of various test organisms for growth (4, 19). In addition to those occurring in nature, B_{12} -like vitamins are obtained experimentally by bacterial synthesis of a specific analogue when the organism is presented with factor B and an appropriate heterocyclic nitrogenous base (13, 18, 36, 42).

Recent findings (43) demonstrate that the soil bacterium, Type III, No. 38 (29) (designated "Lochhead 38") degrades and synthesizes cyanocobalamin and its congeners. Among the in-

tracellular factor B-containing vitamins that have been identified as products of metabolism are: factor B, cyanocobalamin, and the benzimidazole-containing analogue of cyanocobalamin (44). In addition, factors C_1 and C_2 (26) occur in significant amounts. It seems likely that Lochhead 38 also carries out these decomposition and synthetic reactions in soil, and that marine organisms with similar biosynthetic capacities exist.

Studies in progress (44) suggest the contribution of Lochhead 38 to the supply of B_{12} -like vitamins in soil may be qualitatively and quantitatively flavored by the proportions of nitrogenous bases, or their precursors, that are available. Riboflavin is typical of soil constituents that occur in relatively high concentrations (8). The utilization of riboflavin by bacteria as a source of the 5, 6-dimethylbenzimidazole moiety of cyanocobalamin was initially suggested by Wooley (62) and subsequently demonstrated experimentally by Ford and co-workers (18). Unpublished data (44) indicate that Lochhead 38 also transforms riboflavin to 5, 6-dimethylbenzimidazole which it then uses in the synthesis of cyanocobalamin. In the course of this directed synthesis, the production of several of the pseudo-vitamins is modified. Thus, when graded concentrations of riboflavin are fed to Lochhead 38, a proportionate increase in the production of cyanocobalamin is observed. This is often accompanied by an inversely proportionate decrease in the synthesis of the benzimidazole-containing analogue. Concomitantly, there are parallel, but less dramatic increases in the output of several other pseudo-vitamins.

The effect of riboflavin on the production of vitamin B_{12} -like factors in samples of detritus (*i.e.*, the suspended matter of a water sample which is retained by a millipore filter: mixed microbial populations, silts, etc.) was also examined (53). Higher levels of vitamin B_{12} -activity, as determined by *E. coli* assay, were found occasionally in aliquots of detritus samples incubated with riboflavin than were found in control samples without supplement. However, the net production of vitamins B_{12} in nature would be governed by the properties of the mixed populations which are present. Thus, it is possible that utilization may at times exceed production, in which case a depletion of vitamins B_{12} or their precursors may occur with an ensuing shift in population.

The distribution of vitamin B₁₂-active substances

In a series of studies conducted in Georgia, the relative amount of vitamin B₁₂-active substances present in detritus was measured (47). The area surveyed is located near Sapelo Island, Georgia. It is separated from the mainland by approximately 4 to 6 miles of extensive salt marsh in which *Spartina alterniflora* predominates. This area contains an elaborate drainage system accessible to the open ocean. Tide levels, at times, exceeded 9 to 10 feet. As a result of tidal action, the rich salt marshes are periodically flooded and drained. Throughout this flushing process, a continual exchange of nutrients occurs between the ocean waters and the marsh lands. This exchange of food substances could involve either the depletion or enrichment of one or the other source or serve to the mutual advantage of both. The ecological effects of these processes are readily noted by comparison of the numbers of plant and animal communities which inhabit a marshy coastal area and a sandy beach. In general, marshy areas support varied populations, whereas sandy regions are relatively unproductive insofar as different forms are concerned.

Microbiological assay procedures (*E. coli* 113-3) were used to measure the vitamin B₁₂ content of detritus harvested from water samples by filtration. Surface water was collected at stations ranging from the open ocean, through a sound, and into a tidal river. On the basis of weight of vitamin B₁₂ per weight of detritus, relatively high values were found in the detritus from offshore waters. The smallest amounts were found in the detritus from the sound waters. Significantly, the highest values of vitamin B₁₂-active substances were obtained from samples collected near the head-waters of the river which meanders through rich *Spartina* marsh. In subsequent experiments, samples were collected during the course of a tidal cycle at one station located near the head of the river. The waters draining from the marsh immediately after slack high water contained detritus richer in vitamin B₁₂-active substances than that found in samples obtained immediately before slack high water and during the ebb tide to low water.

Results of other studies (47) indicate that mixing of muds and sediments by tidal action did not add appreciable quantities of vitamin-rich detritus to the waters. Conceivably there is a more rapid production than utilization of vitamin B₁₂ in the marsh,

and a more rapid utilization than production in the muds and sediments.

Vitamin B₁₂ content of mullet and shark sera

As a sequel to studies on the distribution of vitamin B₁₂-active substances and production by microorganisms, the vitamin content of mullet and shark sera (52) was examined. Values of vitamin B₁₂ (*Euglena* assay) for mullet ranged from 4 to 18 mμg/ml and for shark from 0 to 3 mμg/ml. The significantly higher values for mullet compared to shark serum may reflect physiological differences between these subclasses of fish and possibly different feeding habits. Since mullet or our other fisheries may obtain vitamin B₁₂ in their diet or from their intestinal flora, bacteria isolated from mullet intestinal contents were assayed for ability to produce vitamin B₁₂-active substances. Eight bacteria having different physiological and morphological properties were tested. Results showed that 6 cultures had activity for *E. coli* and 1 culture had activity for *Euglena*. However, cell-concentrates of the bacteria of 2 of 4 supposedly negative cultures had activity for *Euglena*. Thus, the intestinal flora may contribute to the vitamin B₁₂ content of mullet serum.

It would be difficult at this time to review the lengthy literature on the distribution of vitamins B₁₂ in our fisheries. The significance of vitamins B₁₂ to our fisheries and productivity is indicated by its presence in shrimp, oysters, and clams (40), other invertebrates (33, 34), and vertebrates (32, 55).

Vitamins B₁₂: limiting factors in marine environments?

Are vitamin B₁₂-active substances, at times, limiting factors in estuarine and oceanic waters? Lewin (27) and Droop (15) assayed coastal waters and found 5-10 mμg B₁₂/l. We found (47) approximately 1.5-13 mμg/l and these values varied with the load of suspended matter in each sample. Burkholder and Burkholder (5) found 1.3-10.7 mμg/l of water sample. According to Droop (16), levels of 5-10 mμg/l could support the growth of the heaviest known plankton crops. However, Cowey (11) assayed water samples from the North Sea and Norwegian Deep. He recorded values as little as 0.1 mμg/l which are low enough to hold promise of significant spacial and temporal differences (16). Comparison of these figures would indicate that vitamin

B₁₂ may not be a limiting factor in estuarine and coastal waters. Perhaps it may be limiting in these areas during different seasons. It is apparent that more work is needed on the distribution of vitamin B₁₂ in oceanic waters. The observations that coastal and estuarine environments are high vitamin B₁₂-producing habitats (47) are consistent with the greater number of bacteria per unit volume of water found in these habitats as compared to open ocean waters.

Conclusion

Microbiological and biochemical analysis of waters and of the suspended matter of water with its inherent and adhering nutrients promises to provide useful parameters for the oceanographer and marine ecologist. In this paper, part of the vitamin B₁₂ cycle in the marine environment is reviewed. The importance of this family of compounds for the growth of many marine algae which constitute an important element of the food-network has been emphasized. Physical factors such as tidal movements, winds, and currents undoubtedly aid in the dissemination of these essential products of microbial origin. Thus, their presence in any particular area does not signify their production in that area. In this respect, effluents from productive land masses may contribute greatly to the productivity of distant as well as neighboring waters.

Observations on the biosynthesis of vitamins B₁₂ by the soil bacterium, Lochhead 38, together with the results of differential assays of B₁₂-like vitamins in sea-water indicate the importance of vitamins in nature. These observations imply, also, that shifts in the direction of biosynthesis, brought on by changing conditions of nutrient supply, will contribute to the character of the population by virtue of the specific vitamin requirements of organisms constituting the population.

REFERENCES AND NOTES

1. Armitage, J. B., J. R. Cannon, A. W. Johnson, L. F. G. Parker, E. L. Smith, W. H. Stafford, and A. R. Todd: The chemistry of the vitamin B₁₂ group. III. The course of hydrolytic degradations. *J. Chem. Soc.*, 3849-3864, 1953.
2. Ballantine, D., and B. C. Abbott: Toxic marine flagellates; their occurrence and physiological effects on animals. *J. Gen. Microbiol.*, 16: 274-281, 1957.
3. Bernhauer, K., K. Blumberger, and P. Petrides: Activity of vitamin-B₁₂ factor III in pernicious anemia. *Arzneimittel-Forsch.*, 5: 442-446, 1955.

4. Brown, F. B., J. C. Cain, D. E. Gant, L. F. G. Parker, and E. L. Smith: The vitamin B₁₂ group. Presence of 2-methylpurines in factors A and H and isolation of new factors. *Biochem. J.*, 59: 82-86, 1955.
5. Burkholder, P. R., and L. M. Burkholder: Vitamin B₁₂ in suspended solids and marsh muds collected along the coast of Georgia. *Limnol. Oceanogr.*, 1: 202-208, 1956.
6. Burton, M. O., and A. G. Lochhead: Studies on the production of vitamin B₁₂-active substances by microorganisms. *Can. J. Botany*, 29: 352-359, 1951.
7. Burton, M. O., and A. G. Lochhead: Production of vitamin B₁₂ by *Rhizobium* species. *Can. J. Botany*, 30: 521-524, 1952.
8. Carpenter, C. C.: Riboflavin-vitamin B₂ in soil. *Science*, 98: 109-110, 1943.
9. Coates, M. E., and J. E. Ford: Methods of measurement of vitamin B₁₂. *Biochem. Soc. Symposia*, 13: 36-51, 1955.
10. Connell, C. H., and J. B. Cross: Mass mortality of fish associated with the protozoan *Gonyaulax* in the Gulf of Mexico. *Science*, 112: 359-363, 1950.
11. Cowey, C. B.: A preliminary investigation of the variation of vitamin B₁₂ in oceanic and coastal waters. *J. Mar. Biol. Assn. U. K.*, 35: 609-620, 1956.
12. Davis, C. C.: *Gymnodinium brevis* sp. nov., a cause of discolored water and animal mortality in the Gulf of Mexico. *Bot. Gaz.*, 109: 358-360, 1948.
13. Dellwig, H., E. Becker, and K. Bernhauer: Biosynthesis in the cobalamin group. *Biochem. Z.*, 328: 88-95, 1956.
14. Droop, M. R.: Cobalamin requirement in *Chrysophyceae*. *Nature, Lond.*, 174: 520, 1954.
15. Droop, M. R.: A suggested method for the assay of vitamin B₁₂ in sea water. *J. Mar. Biol. Assn. U. K.*, 34: 435-440, 1955.
16. Droop, M. R.: Auxotrophy and organic compounds in the nutrition of marine phytoplankton. *J. Gen. Microbiol.*, 16: 286-293, 1957.
17. Ericson, L. E., and L. Lewis: On the occurrence of vitamin B₁₂-factors in marine algae. *Ark. Kemi.*, 6: 427-442, 1953.
18. Ford, J. E., E. S. Holdsworth, and S. K. Kon: Biosynthesis of vitamin B₁₂-like compounds. *Biochem. J.*, 59: 86-93, 1955.
19. Ford, J. E., and S. H. Hutner: Role of vitamin B₁₂ in the metabolism of microorganisms. *Vitamins and Hormones*, 13: 101-136, 1955.
20. Gunter, G., R. H. Williams, C. C. Davis, and F. G. W. Smith: Catastrophic mass mortality of marine animals and coincident phytoplankton bloom on the west coast of Florida, November 1946 to August, 1947. *Ecol. Monogr.*, 18: 309-324, 1948.
21. Hall, H. H., J. C. Benjamin, H. M. Bricker, R. J. Gill, W. C. Haynes, and H. M. Tsuchiya: A survey for B₁₂ producing microorganisms. *Proc. Soc. Am. Bact.*, 50: 21, 1950.
22. Hodgkin, D. C., J. Kamper, M. Mackay, J. Pickworth, K. N. Trueblood, and J. C. White: Structure of vitamin B₁₂. *Nature*, 178: 64-66, 1956.
23. Hoff-Jørgensen, E.: Microbiological assay of vitamin B₁₂. In: B. Glick, "Methods of Biochem. Anal.," vol. 1, Interscience, N. Y., pp. 81-113, 1954.
24. Hutner, S. H., M. K. Bach, and G. I. M. Ross: A sugar-containing basal medium for B₁₂-assay with *Euglena*; application to body fluids. *J. Protozool.*, 3: 101-112, 1956.

25. Hutner, S. H., and L. Provasoli: A pigmented marine diatom requiring vitamin B₁₂ and uracil. *News Bull. Phycol. Soc. Am.*, 6: 7-8, 1953.
26. Kon, S. K.: Other factors related to vitamin B₁₂. *Biochem. Soc. Symposia*, 13, 17-35, 1955.
27. Lewin, R. A.: A marine *Stichococcus* sp. which requires vitamin B₁₂ (cobalamin). *J. Gen. Microbiol.*, 10: 93-96, 1954.
28. Lochhead, A. G.: Soil bacteria and growth-promoting substances. *Bact. Rev.*, 22: 145-153, 1958.
29. Lochhead, A. G., and M. O. Burton: Qualitative studies of soil microorganisms. XII. Characteristics of vitamin B₁₂-requiring bacteria. *Can. J. Microbiol.*, 1: 319-330, 1955.
30. Lochhead, A. G., and R. H. Thexton: Vitamin B₁₂ as a growth factor for soil bacteria. *Nature, Lond.*, 167: 1034, 1951.
31. Lucas, C. E.: The ecological effects of external metabolites. *Biol. Revs. Cambridge Phil. Soc.*, 22: 270-295, 1947.
32. Masaaki, Y.: Studies on the vitamin B₁₂ of aquatic animals. I. The vitamin B₁₂ content of fishes. *Bull. Jap. Soc. Sci. Fish.*, 17: 389-392, 1952.
33. Masaaki, Y.: Studies on the vitamin B₁₂ of aquatic animals. III. The vitamin B₁₂ content of shellfish. *Bull. Jap. Soc. Sci. Fish.*, 18: 636-638, 1953.
34. Maxwell, B. E.: The distribution of vitamin B₁₂-active substances in some marine invertebrates of British Columbia. *J. Fish. Res. Bd. Can.*, 9: 164-168, 1952.
35. McNutt, W. S.: Nucleosides and nucleotides as growth substances for microorganisms. *Progr. Chem. Org. Nat. Prod.*, 9: 401-442, 1952.
36. Perlman, D., and J. M. Barrett: Biosynthesis of cobalamin analogues by *Propionibacterium arabinosum*. *Can. J. Microbiol.*, 4: 9-15, 1958.
37. Provasoli, L., and I. J. Pintner: Ecological implications of *in vitro* nutritional requirements of algal flagellates. *Ann. N. Y. Acad. Sci.*, 56: 839-851, 1953.
38. Ray, S. M., and W. B. Wilson: Effects of unialgal and bacteria-free cultures of *Gymnodinium brevis* on fish. *Fishery Bull. No. 123*, 57: 469-495, 1957.
39. Rickes, E. L., N. G. Brink, F. R. Koniuszy, T. R. Wood, and K. Folkers: Crystalline vitamin B₁₂. *Science*, 107: 396-397, 1948.
40. Robbins, W. J., A. Hervey, and M. E. Stebbins: Further observations on *Euglena* and B₁₂. *Bull. Torrey Bot. Club*, 78: 363-375, 1951.
41. Robinson, F. M., I. M. Miller, J. F. McPherson, and K. Folkers: Vitamin B₁₂. XVI. Degradation of factor III to 5-hydroxybenzimidazole and derivatives and biosynthesis of factor III. *J. Am. Chem. Soc.*, 77: 5192, 1955.
42. Sanders, F.: Biosynthesis of members of the vitamin B₁₂ group. Ph.D. thesis, Univ. of Tex. Med. Branch, 1957.
43. Sanders, F., and G. R. Seaman: Nature of the cobalamin requirements of the soil bacterium, Lochhead 38. *J. Gen. Microbiol.*, submitted, 1958.
44. Sanders, F., and T. J. Starr: The synthesis of cyanocobalamin by Lochhead 38. (in preparation.)
45. Sommer, H., W. F. Whedon, C. A. Kofoid, and R. Stohler: The relation of paralytic shellfish poison to certain plankton organisms of the genus *Gonyaulax*. *Arch. Path. (Lab. Med.)*, 24: 537-559, 1937.
46. Starkey, R. L.: Interrelations between microorganisms and plant roots in the rhizosphere. *Bact. Rev.*, 22: 154-172, 1958.

47. Starr, T. J.: Relative amounts of vitamin B₁₂ in detritus from oceanic and estuarine environments near Sapelo Island, Georgia. *Ecology*, 37: 658-664, 1956.
48. Starr, T. J.: Ecological roles of microorganisms and the economy of the oceans. A.I.B.S., Proc. 14th Gen. Meeting, Soc. Indust. Microbiol., 1957.
49. Starr, T. J.: Notes on a toxin from *Gymnodinium breve*. *Tex. Rep. Biol. and Med.*, 16: 500-507, 1958.
50. Starr, T. J., and M. E. Jones: The effect of copper on the growth of bacteria isolated from marine environments. *Limnol. Oceanogr.*, 2: 33-36, 1957.
51. Starr, T. J., M. E. Jones, and D. Martinez: The production of vitamin B₁₂-active substances by marine bacteria. *Limnol. Oceanogr.*, 2: 114-119, 1957.
52. Starr, T. J., D. Martinez, and W. Fosberg: The vitamin B₁₂ activity of mullet and shark serum. *Limnol. Oceanogr.*, 2: 111-113, 1957.
53. Starr, T. J., and F. Sanders: Effect of riboflavin on vitamin B₁₂ activity of mixed populations. (unpublished)
54. Sweeney, B. M.: *Gymnodinium splendens*, a marine dinoflagellate requiring vitamin B₁₂. *Am. J. Bot.*, 41: 821-824, 1954.
55. Tarr, H. L. A., B. A. Southcott, and P. W. Ney: Vitamin B₁₂-active substances in fish products. *Food Tech.*, 4: 354-357, 1950.
56. Waksman, S. A., M. Hotchkiss, and C. L. Carey: Marine bacteria and their role in the cycle of life in the sea. *Biol. Bull.*, 65: 137-167, 1933.
57. West, R.: Activity of vitamin B₁₂ in addisonian pernicious anemia. *Science*, 107: 398, 1948.
58. Williams, R. T.: The biochemistry of vitamin B₁₂. *Biochem. Soc. Symposia*, 13: 1-123, 1955.
59. Wilson, W. B., and A. Collier: Preliminary notes on the culturing of *Gymnodinium brevis* Davis. *Science*, 121: 394-395, 1955.
60. Wilson, W. B., and S. M. Ray: The occurrence of *Gymnodinium brevis* in the western Gulf of Mexico. *Ecology*, 37: 388, 1956.
61. Wood, E. J. F.: The significance of marine microbiology. *Bact. Rev.*, 22: 1-19, 1958.
62. Wooley, D. W.: Selective toxicity of 1,2-dichloro-4,5-diaminobenzene: its relation to requirements for riboflavin and vitamin B₁₂. *J. Exp. Med.*, 93: 13-24, 1951.
63. ZoBell, C. E.: Marine Microbiology. Chronica Botanica Co., Waltham, Mass., 1956.

^a Presented (48) in part at the A.I.B.S. meeting, Stanford Univ., 1957. Accepted for publication November 20, 1958.

^b The University of Texas Medical Branch, Department of Preventive Medicine and Public Health, Virus Research Laboratory and Department of Internal Medicine, Galveston, Texas.